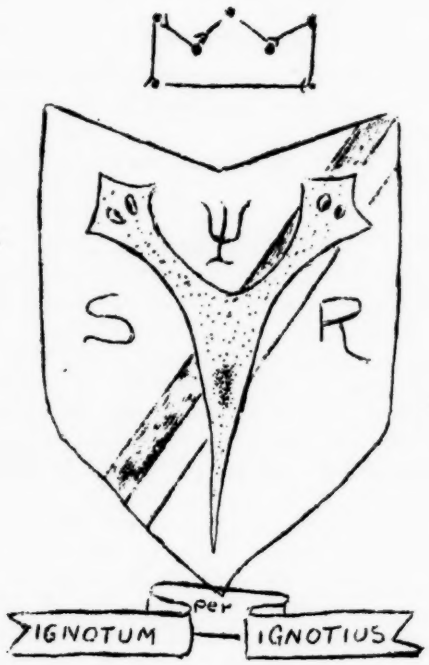

THE WORM RUNNER' S DIGEST

Planaria thou art cool
so gray in spots, so way outside.
so squirm and flat, worm, but crosseyed
No Crypto-Freudian art thou, Clyde,
you be the new Pavlovian tool.
Planaria thou art cool.

Planaria you dig this school--
the scene reads light, shock, contract
then bug you in the middle back
and twin you, man, like Kerouac.
and play that crazy scene in dual.
Planaria thou art but cool.

--Joe Jacobson

A special reprint issue of:
Volume I, Number 1 (November, 1959)
and
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THE WORM RUNNER' S DIGEST
An Informal Journal of Comparative Psychology
Published Irregularly By
The Planaria Research Group
Department of Psychology
The University of Michigan
Ann Arbor, Michigan

November, 1959 THE WORM RUNNER'S DIGEST Vol. I, No. 1

An Informal Journal of Comparative Psychology

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Cover by Reeva Jacobson

During the past few months, as some of our initial conditioning studies with planarians appeared in print, we began receiving requests for detailed information on our procedure and apparatus. We have also had inquiries on where planarians may be obtained and how they should be cared for. At first we tried to answer all such letters individually, but it soon became apparent that most people wanted pretty much the same information. We decided to put as much of our knowledge as we could down in dittoed form and make it available to anyone who wanted it. This process has taken us many weeks longer than we had hoped, but here it is--Volume I, No. 1, of the Worm Runner's Digest. Further issues will appear irregularly, as they seem needed and as we have further information to impart.

As we were preparing this initial issue of the DIGEST, we decided to include informal presentations of results from our own and other laboratories, as well as information on what other people were planning to do in the way of research with planarians. We have hopes that the DIGEST may act as a sort of clearing house, passing along data to those interested in such long before final results of the various studies can appear in the scientific journals. Perhaps we can also encourage correspondence among those of you scattered about the world who would like to work with this fascinating animal.

This issue of the DIGEST contains articles by various members of the Planarian Research Group in the Department of Psychology at Michigan. We have no intention of monopolizing the pages of the DIGEST, and hope that we can prevail upon many of our readers to "do a little article" for the DIGEST on some aspect of their work with planarians (or other lower organisms). We also wish to apologize if occasional hints of levity creep into the pages of this austere scientific publication. We enjoy life, and we think that working with worms can be fun as well as a worthwhile research endeavor. Our efforts in the lab are no less rigorous for our being able to joke about our results.

In return for our sending you the DIGEST, may we ask one favor? We want to know what you are doing, what results you get, what difficulties you encounter, what theories you are postulating. It took us two years to learn how not to run worms before we painfully learned the right ways to condition them and keep them alive and happy. We can probably all save each other considerable time and effort if we keep in touch, for science thrives on speedy communication.

We wish you good reading, and good research! Ignotum per ignotius!

James V. McConnell

TITLE: Apparatus

AUTHOR: Allan Jacobson
Planaria Research Group
University of Michigan

I. Housing

The planaria are housed together before experimentation in glass aquaria, measuring 8"L by 5"W by 5"D. Water in the aquaria is continuously aerated and maintained at room temperature. During experimentation each worm is housed in its own finger-bowl and the water is changed every three days in the process of feeding.

II. Transporting of worms

Transporting worms from aquaria to experimental apparatus and back is accomplished by means of an ordinary glass pipette, of which the tip end has been removed to prevent damage to the worms.

III. Experimental apparatus

Consists of trough, lights, inductorium, switch for lights, and switch for shock.

(a) Trough - consists of a V-shaped groove, 12"L and ½"D, cut into a white plastic block; the sides of the groove are sloped to meet at a 90-degree angle. The dimensions of the plastic block are 14"L by 2"W by 1"D.

A metal insert is placed in each end of the trough to receive the leads which go to the inductorium, thence to a low-voltage power supply unit, and finally to an outlet. The ends of the inserts are shaped to fit snugly into the groove and thus block the ends of it.

We have also used semi-circular troughs with good success. They can be made rather easily by cutting a plastic tube in half lengthwise, and mounting electrodes at the end. Ed Ernhart (Washington University, St. Louis) reports that for mazes, he gets the best results by cutting semi-circular grooves in clear plastic. When he used a square-shaped groove, the worms crawled on the sides of the grooves rather than on the bottom and this made their behavior at the choice-points rather difficult to judge. After all, worms crawling on the sides of a groove wouldn't know whether they were turning left or right--rather they would be turning up or down.

(b) Lights - two lamps are placed 6" above the trough, each holding a 100-Watt frosted bulb. The center of each bulb is above approximately the half-way point between the center of the groove and each end of the groove, respectively.

(c) Inductorium - regulates intensity of shock.

(d) Switch for lights - is an ordinary wall light-switch, telegraph key or microswitch.

(e) Switch for shock - is a telegraph key

The trough, lamps, and inductorium are mounted on a wooden frame, which is securely clamped to the table.

The two switches are attached to a board so that one person can operate them. This board is used away from the table, so as to prevent vibrations of the trough.

NOTES

1. A Harvard stimulator was found less dependable than the inductorium as a regulator of shock intensity. With a regular stimulator, shock of sufficient intensity to cause contraction will harm the worm; but, probably due to the "make-break" nature of the inductorium wave shape, an inductorium works very well.

2. A semicircular trough has poorer visibility than the V-shaped groove.

3. In a square trough the worm tends to swim along the side.

TITLE: Planaria Running Procedure

AUTHORS: Paul and Gretchen Cornwell
Planaria Research Group
University of Michigan

1. Fill trough with pond or river water.

2. Take S out of aquarium and transfer it to experimental trough.

3. Let S move about in trough 5 minutes before beginning experiment trials.

4. When S is gliding parallel with the direction of the trough, the light (CS) is turned on. At the turning on of the light, E begins counting: "one thousand one, one thousand two". When E gets to "two" he switches on the shock (US). At the "three" E turns off both CS and US.

5. E records the responses of S to: 1) the CS, and 2) the US. (See "Judgment of responses", below.)

6. E starts stop watch.

7. After one minute E stops watch.

8. The first time S is gliding in the longitudinal direction, E begins another trial.

9. Thirty (30) such trials are run each 24 hours.

Commonly Encountered Problems

1. If S is gliding parallel to the direction of the trough, but is gliding on the surface tension of the water (instead of the bottom or side of the trough), the experiment trial is not begun until S resumes gliding on the bottom or side of the trough.

2. If S stops gliding wait one minute. Then, if still not gliding, gently squirt some water over S with the medicine dropper. Allow 30 seconds to then elapse before administering the next trial.

3. If procedure #2 has to be used ten times during one day, the following time that S stops and remains stopped allow 5 minutes to elapse. If S is still not gliding put him back in the aquarium and do not run him again until the following day.

Judgment of Responses

<u>Unconditioned Response</u>

a. seems to be partly a function of US (shock)
b. ranges from S stopping movement to S contracting violently, curling up in a ball, and then protruding the pharynx.

Unconditioned Response (cont'd.)

Recording symbol	Behavior
U_1	1. stop 2. stop and slight withdrawal of anterior end
U_2	3. smooth contraction of entire body 4. strong contraction of entire body 5. contraction of entire body in which both anterior and posterior rise off substratum
U_3	6. violent contraction a. after contraction S often curls up in a ball--sometimes "throws out pharynx" b. after contraction S often does not immediately resume gliding - instead S often exhibits ditaxic locomotion

Conditioned Response

Recording symbol	Behavior
C_1	1. stop 2. stop with slight withdrawl of anterior 3. anterior raised off substratum (also all doubtful responses)
C_2	4. smooth contraction of entire body
C_3	5. strong or violent contraction of entire body
R_1 or L_1	6. anterior turned to right or left - not abruptly
R_2 or L_2	7. anterior turned to right or left abruptly

Note: Although a statistical analysis has not been run on the frequency of the various CR's, some worms do have a tendency to form a turning CR while others are "contractors".

Feeding and Water Changing

1. Every other day the water is changed in each aquarium. This is done most conveniently if it is changed during the time S is being run. Aerated pond or river water is used.

2. About twice a week each S is fed fresh brine shrimp. These are added to the aquarium <u>after</u> S has been run. After two hours the water and uneaten shrimp are poured off, and fresh water is added.

DATA SHEET

Animal #_____ Length_____ Date_____ Page #_____

Trials			Trials			Trials		
1			26			51		
2			27			52		
3			28			53		
4			29			54		
5			30			55		
6			31			56		
7			32			57		
8			33			58		
9			34			59		
10			35			60		
11			36			61		
12			37			62		
13			38			63		
14			39			64		
15			40			65		
16			41			66		
17			42			67		
18			43			68		
19			44			69		
20			45			70		
21			46			71		
22			47			72		
23			48			73		
24			49			74		
25			50			75		
Time Ended								
Total								

TITLE: The Feeding and Care of Planaria (Dugesia tigrina and dorotocephala)

AUTHOR: Margaret Clay
Planaria Research Group
University of Michigan

It has long been imagined that to properly care for laboratory animals one needed only to cage them, nourish them, and avoid gross temperature changes. The prime example of animal deprivation of this sort is the laboratory rat, who lives always in essentially a deprived environment and therefore is less healthy and downright dumb compared to his natively reared cousin, the barn or river rat. Planaria, though much further down the phylogenetic scale, are also living beings, and as such may be counted on to respond positively to tender, lovin' care. That is, they'll have more get up and go, will learn better, and (classified information): are more likely to do what you tell them you want them to do before you run them. This leads to more clear-cut experimental results, a positive reinforcement for homo sapiens scientifica.

A good rule for raising any living thing is: when in doubt, ask the beastie. That is, as close an approximation to his native environment as you can get is usually the most satisfactory arrangement. This isn't always easy or practical to do, but with planaria, fortunately, it can come close to being accomplished. Natively, they live mostly in not too active streams, lakes, and ponds, that vary in temperature but are always at least on the cool side, and preferably that contain rocks in not too deep water for crawling onto the under side of--out of the hustle and bustle and light. They dine when hungry--if food is available then--preferring either dying or fresh killed animals of a wide variety. I've seen them feed on microscopic or definitely macroscopic (½ inch long) brine shrimp, daphnia of all sizes, snails, and even guppies which had been wounded and weren't in much shape to fight back. They appear to be sort of lazy (and perhaps a wee bit cowardly at times) in that they avoid anything with too much energy, preferring to wait till it tires, gets mixed in the mucous they've secreted, or just plain dies, before they glide up onto it and begin their meal. But if it's been dead too long and is no longer fairly fresh, they'll have nothing to do with it, ordinarily. (There's one in every crowd, you know, that will do what you say they won't, just to foul you up.)

In every lab, of course, it will be necessary and sometimes desirable to modify things a bit, and ours is no exception. But we recommend getting as close to natural conditions as possible first, and taking your modifications from there. Begin with stock gleaned from nature rather than inheriting someone else's laboratory mistakes. We keep our stock after arrival in an aerated aquarium, in water which we bring in by carboy from a local river. The aquarium contains half a dozen fairly smooth rocks, collected by our more naturalistically inclined members from some of the local farm and wood lands. Too much other paraphernalia gets in the way when cleaning the tank or searching for worms. These rocks are fortuitous both for the planaria and for us. They feel at home under them, and when we want stock for experimentation, we simply pick a rock out of the tank, set it in a small bowl of river water, and they crawl off onto the sides of the bowl where we can pick out what we want at our leisure. Every day or so, we drop a liberal quantity of small brine shrimp or living daphnia (or occasionally a batch of small snails) into the aquarium.

Water in the aquarium is replaced as it evaporates. We maintain it at a level of roughly three to six inches deep. Every three or four days, debris begins to accumulate on the bottom of the tank. This can be removed by suction with a pipette consisting of a fairly long glass tube (12-18 inches) with a large rubber bulb at one end, the other end fire-polished. If you don't scrape the bottom of the tank as you move the pipette along, but keep the end of the tube just off the bottom, you will collect almost without exception debris rather than planaria. If you find that the debris remains suspended in the water rather than collecting in clumps on the bottom, you're aerating the tank too well--cut down on the bubble flow. Seamless aquaria are highly desirable, by the way, since the worms like to crawl into seams as well as under rocks.

As for cleaning the aquarium, this is necessary, but can be overdone. Since the worms secrete a mucous on the glass as they glide about, this will eventually accumulate, and debris will tend to stick to the sides and bottom. There appears to be a direct inhibiting effect of this mucous on regeneration, so if you want your stock to drop tails and multiply often, clean the tank by draining all water and washing with dilute HCl every week or two. If you want them to simply grow big and strong till you use them, this is not particularly essential, depending on the size of your tank, how well you clean it with the pipette, and how rapidly you use up the stock. With each new batch from the great outdoors, however, a fresh tank should be set up. (Cleaning with HCl rather than detergent, or thorough rinsing after detergent use, is important, as deposits of this sort in the water make the beasties sick and sometimes dead.) (By the way, stock ordered from most biological supply houses has been collected rather than raised in captivity, and is usually quite satisfactory. We obtain our planaria from the Michigan Scientific Company, Jackson Highway, Ann Arbor, Michigan. If you ask them to, they will send you the largest animals available. These will be mostly D. Tigrina and D. Dorotocephala. Large Maculata can be obtained from the Turtox people in Chicago. We have been told (but cannot swear) that the Carolina Biological Supply people have the smallest worms in the world for sale. We prefer the larger ones. Easier on the eyes, if nothing else, and much easier to work with if you're interested in studies on regeneration.)

Though it is not essential, we try to select our stock for experimentation at least a few days before we plan to use them and put into small finger bowls (preferably white rather than clear glass--they show up better). There we can begin to control their diets more rigorously and get them used to "the experimental environment". Fifteen to thirty worms can be kept in a four inch finger bowl without crowding, though each worm is maintained separately after experimentation begins. Since these are not aerated, nor do they contain near so much water or surface area as aquaria, we change the water in them at least every two days, and most certainly within twenty-four hours after feeding. In most conditions, we feed these worms every two days by depositing in the finger bowls about 1-2 mls. of newly hatched brine shrimp which have been washed in fresh water. Once running, if fed at all (this depends on the conditions of your experiment--if necessary they'll live nicely for a couple of weeks without food), a couple of times a week is often enough, and it's usually desirable to remove the food after they've had a couple or three hours to feed. Planaria feed by attaching their pharynxes to the food and secreting digestive juices onto it to soften it up so they can ingest. This means that the surrounding water gets some of these juices, so cleaning after feeding is a must unless the medium is in sufficient abundance to allow for natural diffusion, etc. without much affecting the total concentration in the water (as

with a fair-sized, pretty full aquarium that's being aerated). Since they are negatively phototropic (a fancy way of saying they're more comfortable in the dark), you're doing them a kindness to put them in a dark room or under a box while they feed. (This, too, of course, may depend upon your experimental design.)

Planaria are carnivorous but will eat a wide variety of animal matter. Many labs feed them liver or chopped earthworm and this maintains them satisfactorily. We feel they should be required to work at least a little for their food, so try to give them at least occasional live food, particularly while they are in the aquarium. We also vary their diets some, since who knows what holds most nutritious food value for a planarian? One word of caution: if fed on egg yolk, they will color up beautifully so that one can observe their internal organs rather well, particularly the digestive tract. They will also starve, since they cannot digest and get nutrients from it.

The most staple item in the diets of our star boarders is brine shrimp. These can be raised simply, economically, and efficiently right in the lab, and, though they are raised in salt water, will live for a short time after being rinsed in a fresh water bath, and our planaria love them. Brine shrimp eggs can be ordered from biological supply houses (or sometimes purchased at the local pet or tropical fish shop). A very few eggs go a very long way. We set them up in shallow glass trays (pyrex dishes suitable for baking a one-layered cinnamon coffee cake are <u>just</u> fine). The trays are first filled almost to the top (depth of 1 to 2 inches) with seasoned tap water (meaning that it has been sitting in a container for at least 24 hours, to allow evaporation of undesirable chlorine, etc.) to which uniodized salt has been added. The instructions on the eggs say it should be ocean water concentration. We get quite satisfactory yields by adding about 20 cc's of saturated salt water to every 500 cc's of tap water. The shrimp eggs are then scattered over the surface of the water about as you would sprinkle cinnamon over the top of the coffee cake, though perhaps not as profusely. About ½ teaspoon sprinkled over a surface area of approximately 8 x 10 inches gives the best yield. The eggs will begin hatching within 24 hours, and at the end of about 48 hours the young brine shrimp can be collected in quantity. If a light shaded to all but one corner of the tray is left on constantly during the hatching process, they will collect in this corner as they hatch, making for ease of collection.

We have found that the simplest way to collect them is with a long-stemmed pipette with a finely drawn tip. From the pipette we transfer them into 15 ml. centrifuge tubes. These are allowed to stand in a refrigerator, and the shrimp settle to the bottom of the tube as they die. A good yield for a single tray this size will fill a centrifuge tube about 3½ ml. full when the shrimp have settled. The excess salt water can then be drained from the top of the tube with the pipette and fresh water can be added. Refrigerated this way, they will stay fresh for several days. If there's no refrigerator handy, standing at room temperature will accomplish the same purpose, but will take longer and give a less efficient yield which won't stay fresh as long. If you prefer to raise the shrimp to adulthood they should be transferred upon hatching from the trays to aquaria containing about the same (or slightly greater) concentration of salt in seasoned tapwater, but with the water at a level of about 4 inches or better. Brewer's yeast, lightly sprinkled on the surface of the water every three or four days, will serve as nourishment. If the shrimp are not crowded, they will grow to about ½ inch within a couple of weeks and then live a week or two longer before they die.

The next simplest and most effective food to raise is daphnia, commonly called the water flea. These are actually small crustacea that live in stagnant pools of fresh (i.e., non-salt) water. They can be raised with minimum effort in a small fish bowl or aquarium in either of two ways. If you have a good source of sunlight, set the aquarium containing pond water of some sort in the light where it will begin to turn green (the pond water, not the aquarium) after a few days, from algae growing on it. Put anywhere from three or four to a couple of dozen daphnia into it, and you'll have started a self-perpetuating system which will go on indefinitely if kept at temperatures above 18 degrees and below 28 degrees centigrade. They reproduce themselves at a phenomenal rate and can be removed from the water with a small plankton net, or any net of very fine mesh, whenever needed. Beyond seeing that the water in the tank doesn't dry up and perhaps occasionally adding more "green" water, they need no care at all. (They live by eating the algae.) If your supply gets low, let it sit for a few days before taking any more daphnia out for worm-feeding, and presto, there'll be hundreds of them again.

If, like ours, your lab is in a subterranean cavern where sunlight doesn't penetrate, you won't be able to keep the water green enough to feed them. A perfectly satisfactory alternative is to put into your tank with the daphnia a few snails. Then every day sprinkle a little Pablum on top of the water. The snails will eat the Pablum, and the daphnia will eat the bacteria from the snail droppings and all will flourish. Snails, too, are phenomenal reproducers, so soon you'll have lots of little snails in your tank along with the daphnia. Thin these out occasionally by throwing them into the planaria tank. Planaria love snails, too, and it gives them another variant of diet with little additional cost and effort.

If you follow these directions faithfully and everything dies, try again, using your own common sense variations. It took Mother Nature thousands of years to really get living things growing successfully, you know, and even with her help, it may take you a few weeks to catch on.

TITLE: Results of Studies of Planaria

AUTHOR: Reeva Jacobson
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University of Michigan

Planaria are excellent subjects for studies of learning, for they are the lowest animals on the phylogenetic scale to possess a true synaptic-type nervous system and powers of complete regeneration.

Around 1900, flatworms were used extensively in biological research (1, 7, 9). One of the facts discovered is that planaria are negatively photosensitive, thus responding to direct light by moving away from it. Hovey (4), by partially extinguishing the locomotor response to light in flatworms, reported behavioral modification known as "negative adaptation" of innate sensory-motor reflexes. When the subjects began to move following exposure to light, Hovey touched the animals' heads, which served to stop their motion temporarily. The number of touches used in a single trial decreased markedly with continued training, but no worm could be trained to stay motionless in light for more than five minutes.

In 1955, Thompson and McConnell (8) reported the establishment of classical conditioning in planaria. Using light as the conditioned stimulus (CS) and electric shock as the unconditioned stimulus (UCS), they found that after 150 trials, the experimental subjects would respond to light alone significantly more times than would control subjects.

McConnell, Jacobson*, and Kimble (6), aware of the regenerative power of the planarian, attempted to find its "locus of learning". Naive animals were subjected to a conditioning procedure similar to that employed in the above mentioned study. Once the conditioned response (a longitudinal contraction of the body) had been well established, the animals were cut in half and allowed to regenerate. The experimenters allowed four weeks for regeneration and then retrained each section to the original criterion of conditioning (23 conditioned responses in 25 consecutive trials). It was discovered that both the head and tail sections showed equal and highly significant retention of the conditioned response. (This study was supported in part by a grant from the Horace H. Rackham School for Graduate Studies, and in part by a grant from the University of Michigan Research Institute.)

Gretchen Cornwell used a method similar to the above studies, with the substitution of vibration as the UCS. She found that the subjects could be conditioned, but showed less retention over time. In a subsequent study, she varied the procedure by using off-set of light as the CS. The light was on between trials and turned off during the three second trial. Shock (UCS) was applied during the last second of the trial. The animals "learned" as before. Some subjects were housed in the light and others in the dark between the daily trials and this did not have an effect on conditioning. (This study was supported in part by a grant from the University of Michigan Research Institute.)

This year, 1959, Jacobson+, McConnell and Maynard (5) demonstrated that planaria formed of completely regenerated tissue, but of tissue originating from conditioned subjects, demonstrate retention of the conditioned response.

*Allan L. Jacobson +Reeva Jacobson

Native planaria were cut (behind the pharynx) and the tail sections discarded. The heads were rapidly conditioned to criterion, using the method reported by McConnell, Jacobson and Kimble. Following training, the head sections were allowed to grow new tails. These "second generation" organisms were then cut in half and each allowed to regenerate into complete "third generation" subjects. They found significant savings in the "third generation" heads and the tails as compared to the original training of the controls, or of the "first generation" heads. There was somewhat less retention of the response in the tails as compared with the heads. (This study was supported in part by a grant from the University of Michigan Research Institute, and in part by a grant from the National Institute of Mental Health.)

As a result of these experiments, it has been theorized that the learning which takes place in planaria is related to a chemical change in the nervous system. The chemicals facilitate learning as well as acting as organizers determining the development of regenerating nerve cells.

Ernhart and Sherrick, at Washington University in St. Louis (3), have demonstrated that planaria can learn a two-unit T-maze. The subjects were placed in a brightly lit maze. By making the correct turn they could enter the darkened goal box (darkness served as the reward). After reaching criterion (three consecutive errorless runs), the planaria were cut in half and allowed to regenerate. Both "second generation" head and tail exhibited significant and equal retention of the maze learning.

Samuel Cummings and Carl Moreland, of Kenyon College (2), used weak vibration as the CS and shock as the UCS. They found that in 150 trials the subjects responded to vibration alone about 25% of the time. In continued trials, the response level dropped to about 10%, as compared to the control group which received vibration alone and in 250 trials responded 25% of the time. It was suggested at APA, where these results were reported, that the experimenters might have been getting conditioned inhibition. However, this is a conjecture, and Cummings and Moreland feel that their study raises grave questions as to whether or not planaria can be conditioned.

John Caylor, University of California (Riverside Campus); Bill Corning, University of Rochester; and Jerry Bernstein, State Teachers College (Clarion, Pennsylvania) report that they have been at least partially successful in replicating some of the earlier work done with planaria.

This year, at the University of Michigan, studies of planaria will include effects of radiation on learning and memory, effects of extinction and partial reinforcement, and effects of grafting of parts of conditioned subjects onto naive animals. (These studies will be supported by research grants from the National Institute of Mental Health and from the Atomic Energy Commission.) At the University of Rochester, Roy John and Bill Corning are involved in studies of the neurophysiology and histology of planaria. They are attempting micro-electrode implantation, so that electrical activity in the nervous system during learning might be recorded. Jay Boyd Best, at Walter Reed Institute in Washington, D.C., is testing approach-avoidance learning in planaria.

We hope that many more experimenters will join us in investigating learning and memory in the cross-eyed flatworm.

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